PERSPECTIVES IN PLANT SEROTAXONOMY[1]

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Abstract

The capacity to view recent data in proper relation to other information, or the ability to correctly judge the significance of facts and ideas, requires a knowledge of both the past mistakes and the forward strides within a discipline. This paper is intended to help the reader formulate perspectives concerning 65 years of plant serotaxonomic research. The discovery that the immune reaction was only relatively specific and that the degree of cross-reactivity was essentially proportional to the degree of relationships between organisms had important implications for comparative systematic serology. It is the specific reactions, between determinants and antideterminants, which provide a measurement of protein similarities. The comparison of protein mixtures, rather than purified single proteins, has dominated taxonomic research because such an approach provides serological overall similarity, and thus a multicharacter comparison. The "antisystematic" reactions have recently been shown to result from variation in the systematic ranges of determinants; and the absorption (presaturation) technique for removing common determinants increases the accuracy of serological placements. The following items were evaluated: antigenic preparations, adjuvants, injection procedures, single versus mixed protein extractions, kind of plant tissue extracted, and the interference of secondary compounds. *Cornus canadensis* and *C. suecica* were found to be serologically very similar. The tested species of the genus *Cornus* were divided into three distinct serological groupings. The serological data support the separation of the Cornaceae and Nyssaceae; and the inclusion of *Camptotheca* and *Nyssa* in the Nyssoideae, and *Davidia* in the Davidioideae, both of the family *Nyssaceae*. *Nyssa biflora* and *N. sylvatica* were serologically very similar; *N. ogeche* and *N. aquatica* were serologically distinct from each other and from *N. biflora* and *N. sylvatica*. *Nyssa ogeche* was the most distinct species of the genus. *Corokia cotoneaster* had very little serological similarity with any of the tested species of the Cornales.

To have the capacity to view recent data in proper relation to other information, or the ability to correctly judge the significance of facts and ideas, requires a knowledge of past mistakes and the forward strides within a discipline. It also requires a degree of knowledge of the individual components as well as the total products resulting from the various component combinations. The author hopes this manuscript will provide the reader with the necessary information and literature citations which will allow the formulation of perspectives concerning 65 years of plant serotaxonomic research.

The "present age" of chemosystematics or chemotaxonomic publications commenced to appear in the early 1950's. The oldest of the "present age" approaches is serotaxonomy and the newest is amino acid sequencing (Cronquist, 1976).

The discovery of serological reactions in Austria via the occurrence of precipitin reactions took place 80 years ago (Kraus, 1897). This discovery provided a new technique which was soon used to aid in the investigation of systematic problems in animals. Within two years after the discovery of the precipitin re-

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[1] I dedicate this publication to the memory of my friend and fellow phytoserologist, Dr. Josef Kloz, who died in his 55th year on October 22, 1976 in Czechoslovakia.

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action the technique was applied to comparative problems by the Frenchman Bordet (1899). This was followed by a series of extensive comparative studies which were conducted with various animals by the Englishman Nuttall (1901, 1904). Thus biologists have known for 75 years that organisms may share antigenic material (substances capable of inducing the formation of antibodies and able to react with the antibodies); and that when they share the same antigenic material in different proportions it is assumed that the organisms are related. At first it was believed that the immune reaction was absolutely specific, that is, that an antiserum would react only with the antigen that stimulated its production. However, Bordet (1899) in conducting research with birds reported that the reaction was only relatively specific and that the degree of cross-reactivity was essentially proportional to the degree of relationships between organisms. It was this early discovery which had important implications for systematics and started the pioneer investigations in the discipline of comparative animal systematic serology.

A large number of animal systematic serological publications have been reported in the bibliography prepared by Leone (1968) and the book edited by Wright (1974). The pioneering work in the United States essentially began with Boyden (1926), and he has continuously contributed to the field of animal systematic serology for 50 years (Boyden, 1973; Wright, 1974). Approximately 550 plant taxa (cultivars through orders) have been included in approximately 160 systematic serological publications since 1950 (Fairbrothers, 1969a; Fairbrothers et al., 1975).

Serology is concerned with the interactions of antigens and antibodies and/or antibodylike substances, the lectins. The term "serology" is often used synonymously with immunology. However, some biologists prefer "serology" because "immunology" has an implication that immunity is concerned in all reactions between antigens and antibodies (Boyden, 1948).

PRINCIPLES OF SEROLOGY

A consideration of a few basic facts related to the biology of the immune response is a valuable aid to understanding the methods and interpretations of systematic serological research.

The term "antigenic" is relative since the response is frequently a property of the route of injection, method of preparation of the antigenic material, and the individual experimental animal used. Thus it is important that details about such items and procedures should always be included as a portion of the methods and materials section of publications. Experimental data have demonstrated the following: (1) There is a certain minimal molecular weight below which substances are not in themselves antigenic. Some of the lower molecular weight substances can become antigenic by mixing them with other substances (adjuvants). (2) Size alone is not enough to guarantee that a molecule will be antigenic. Immunochemists indicate that the specific action is in part due to the rigidity of certain chemical structures (determinants) which are difficult to distort or alter. (3) Usually a molecule must be foreign to an organism to be immunogenic. (4) Substances must be soluble or be able to be broken down

into soluble antigenic components before being capable of inducing antibody formation. (5) Too much antigenic material may cause immunological paralysis (i.e., cannot be immunized). There appears to be a balance between stimulation and paralysis for each antigenic material. (6) Many proteins have been found to be immunogenic, and the best known immunogens are the proteins with molecular weights of 40,000 or more (Abramoff & La Via, 1970).

Of the several kinds of serological reactions, the precipitin reaction has been used most frequently in plant comparative serological investigations. It is a relatively simple reaction capable of being applied to the comparison of the soluble protein antigenic material extracted from all kinds of plants. Microcomplement fixation has proved valuable in animal systematics (Champion, et al., 1974), but has had practically no application in comparable plant research. Serological research can be conducted employing quantitative precipitation, precipitin techniques in solutions, or by various qualitative precipitation techniques in gels. The serological characteristics of proteins are linked with the primary structure of the molecule. The reaction is concerned with points on the molecule (determinants) which are capable of initiating the production of immuno-globulins only in certain cells of animals (not plants). These immuno-globulins possess properties accounting for the bonding to the respective protein reaction position. Thus the serological characteristics of the protein are found in the determinants, which are restricted to certain positions of the molecules.

A fairly accurate estimation of the size of determinants has been obtained from protein fractionation experiments designed to detect the smallest molecule fraction still capable of an immunological response. Arnon & Sela (1969) and others have demonstrated that the active antigenic regions of proteins are composed of 10–20 amino acids.

Systematists and taxonomists are interested in the comparison of antigenic determinants from various taxa. It is the specific reactions between determinants (antigens) and antideterminants (antibodies) which are valuable because they provide a means for the measurement of protein similarities.

Methodology

When deciding the type of antigenic preparation, the process of denaturation, which means structural changes with concomitant loss of biological properties, must be taken into account. Protein antigens are not equal in susceptibility to denaturation. However, all such changes result in some loss of original specificity.

The use or nonuse of adjuvants (Freund's, in our experiments) to increase the level of an immune response (immuno-enhancement) is discussed in systematic serological research. The purpose of an adjuvant is to heighten and prolong the immune response; and the value of this additional material must be judged for each antigenic material. This means its use or nonuse should be decided after experimentation.

The effect of injection procedures on the systematic reaction range has been tested by various experiments. One of the very early reports indicating that longer immunization periods extend the reaction range was conducted with a *Zea mays*

antiserum (Magnus, 1908). Lake et al. (1914) using purified seed protein material in contrast to crude seed extracts as used by Magnus also found that a longer injection period extended the reaction range of the antisera. Several recent publications, with both plant and animal antigens in the form of purified or mixed reagents, indicate that the systematic reaction ranges of antisera are extended by injections continued until a maximum reaction is reached (Boyden, 1971, 1973). Such experiments indicate also that long continued immunization is likely to induce the formation of greater proportions of cross-reacting antibodies, which will reduce the discriminating capacity of the antiserum. Antisera derived from several and long injection periods may reach a higher level of "fidelity" to mixed antigens, and reveal information of value in systematic research (Moritz, 1964). Leone (1952) demonstrated that longer immunization periods tend to cause a lower discriminating capacity.

Therefore, the number of injection series used should be stated so the reader can make proper comparisons. The use of a combination of "short" and "long" injection series may produce the largest amount of data for comparative serological investigations. This should not be considered experimental "manipulation" because it is merely using serological techniques to the fullest advantage based upon our present knowledge of the immune response.

There are two main approaches to serological research: (1) comparison of single proteins, or (2) comparison of protein mixtures. The second approach has dominated taxonomic research (Moritz, 1964; Fairbrothers, 1968; Jensen, 1974a). An example of the comparison of a single protein is best illustrated by the research with phaseolin obtained from *Phaseolus vulgaris*, and the ribulose-1,5 biphosphate carboxylase ("fraction I") found in green plants. Data obtained from such research indicates that the systematic ranges of determinants vary. Some determinants are found throughout the plant kingdom, while others have a very restricted distribution. In general, the low taxonomic yield from single protein investigations has not justified the large preparation effort required (Jensen, 1974a). Serological comparison of protein mixtures which were extracted from seeds, pollen, spores, tubers, or leaves is most common. The results provide a serological overall similarity and thus a multicharacter comparison. Researchers working with plant protein extracts also find tannins, saponins, alkaloids, lipids, and/or polysaccharides, which may have to be inactivated, reduced, or eliminated by diverse extraction procedures.

When using a pure protein, only a very limited number of determinants can be compared. In contrast, when using mixtures of proteins, data from many different determinants are tested, and thus the chances of being misled in terms of serological correspondence are lessened.

"ANTISYSTEMATIC" REACTIONS

A phenomenon which has been designated "antisystematic," "asystematic," or "unexpected" cross-reactions has only very recently been placed in proper perspective (Moritz & Rohn, 1956; Frohne et al., 1961; Moritz, 1964). Jensen (1974a, 1974b) indicated that these terms are no longer used by the above authors because past usage assumed serological convergence, which has been shown not to

be the causative agent for such responses. Moritz (1964) included several reports which illustrated his designated "antisystematic" reaction. He also indicated why such reactions were specific serological reactions, and not some kind of non-specific effect, since they disappeared when presaturation experiments were conducted.

The practice of some researchers during the last several years of designating cross-reactions with a wide systematic range as "antisystematic" should be discontinued. Such wide-range reactions are the result of certain determinants being widely distributed in the plant kingdom. In other words, they should be considered as reactions of determinants that are widespread and remain relatively constant. One such determinant has been demonstrated by the serological research with Fraction I Protein (ribulose-1,5 biphosphate carboxylase) extracted from the tissue of green plants. The serological partial identity detected between wide-ranging taxonomic groups has been shown to be the result of parts of the protein structure unaltered in the course of long periods of evolution (Sugiyama et al., 1969; Jensen, 1974a, 1974b). Thus we now know that the systematic ranges of determinants do vary, and sometimes protein molecules carry several determinants which reveal partial serological identities.

The understanding of the above reactions is important because it has shown the value of the presaturation (absorption) technique for removing common determinants and leaving only those systems specific for each taxon compared, thus providing both a more accurate serological placement and measure of the relative similarity.

The use of various techniques to remove nonspecific reactants which react with normal rabbit serum (NRS) has become standard practice in present-day plant serological research. We now know such responses often come from serological reactions resulting from the presence of lectins. Lectins can be removed by hemagglutination techniques and thus be prevented from interfering with normal serological reactions (Lee & Fairbrothers, 1972).

Thus in recent years experiments have provided answers to some of the former perplexing problems associated with plant systematic serological research. This has been very valuable and allowed the continued development of such research. It also assures that a larger spectrum of species can be compared by using the techniques which are now available, and our percentage of accuracy in terms of serological placements, is continuing to increase.

History of Plant Serology

As with animals, serological techniques were used in plant systematics and taxonomy soon after discovery. Mez of the Botanical Institute, University of Königsberg, Germany, conducted such research with his students and colleagues from 1911–1936 (Mez & Gohlke, 1914; Mez, 1922). The "Königsberger Serodiagnostik Stammbaum" (phylogenetic tree) was the climax of years of research (Mez & Ziegenspeck, 1926). This group was known as the Königsberg Serological School, in contrast to the Berlin Serological School which was headed by Professors Gilg and Schürhoff, who conducted research during the 1920's (Gilg & Schürhoff, 1926). These two groups of researchers (schools) conducted a

literary feud which seriously jeopardized the credibility of plant systematic serological research for essentially 25 years. The techniques employed in the early research proved to have several serious flaws. Mez's technique, where he produced an immune serum through the influence of antigen on serum and eliminated the use of living animals as antisera producers, was a serological "disaster." The vast amount of data reported using this procedure was not valid and has been disregarded.

It was Moritz (1928, 1964) and his colleagues at the University of Kiel, Germany, who, working from 1928 until the present, revealed the value of serological research for plant systematics. Jensen, formerly from Kiel, has recently organized another serological laboratory at the University of Cologne. Frohne continues the systematic serological research at Kiel.

In the United States Chester published plant serological papers in the 1920's and 1930's and also prepared a comprehensive critique about plant systematic serology (Chester, 1937). In 1947 Johnson (1951), with his students, started the present United States trend toward plant systematic serological research. It was he who introduced me to the techniques in 1957 after I had joined the faculty of Rutgers University.

In 1953 Urano (1955) started phytoserological investigations in Japan, and S. Sakaguchi and S. Arai have continued this research (Fairbrothers, 1969a). The *Solanum* serological research in Birmingham, England was started in 1955 (Gell et al., 1956). J. Hawkes (Birmingham) and his students (Lester and P. M. Smith) have continued serological research to the present time. In Prague, Czechoslovakia in the late 1950's the husband and wife team, Kloz and Klozova, started and continued serological investigations of *Vicia faba* and other legumes (Kloz et al., 1960). The year 1963 saw the start of another plant serological research center headed by Vaughan in London, and he has continued his multidisciplinary *Brassica* investigations to the present time (Vaughan & Waite, 1965; Vaughan et al., 1976). Cristofolini (1968) has published several reports from his botanical laboratory in Italy since beginning his plant serological research in 1966. The most recently organized plant systematic serological laboratory is under the leadership of Drs. Morozova, Chupov and Kutjavina at the Komarov Botanical Institute, Leningrad, USSR (Fairbrothers, 1975).

INTERPRETING THE RESULTS

Research has demonstrated that extracts of seeds, pollen, leaves, tubers, and spores of vascular plants can be used, if the required extraction procedures are followed (Fairbrothers, 1969b, 1975; Fairbrothers et al., 1975). However, most systematic serological research has included seed material, due to the relatively high concentration of proteins, relative ease of collecting, and relative ease of assuring comparable developmental stages. We are presently pursuing the following two new studies in our chemosystematic laboratory using pollen as the source of protein material: (1) serological investigation of selected amentiferous taxa with Frank Petersen, and (2) a serological investigation of the Corylaceae (Betulaceae) with Friedrich Brunner.

Phytoserological research has provided provocative and valuable data for use in the classification of flowering plants. The numerous examples cited in the evaluation of the contribution of serological data related to Cronquist's and Takhtajan's systems of classification were shown to be significant (Fairbrothers et al., 1975). This publication indicates that such data have contributed in the classification of the following orders, and the placement of families within these orders: Capparales, Caryophyllales, Cornales, Dipsacales, Illiciales, Lamiales, Magnoliales, Nelumbonales, Nymphaeales, Papaverales, Polemoniales, Ranunculales, Rubiales, Scrophulariales, Typhales, and Umbellales. In addition to these orders, significant contributions have also been published for species, genera, and/or tribes belonging to the following families: Ammiaceae, Berberidaceae, Brassicaceae, Caprifoliaceae, Cucurbitaceae, Chenopodiaceae, Cornaceae, Fabaceae, Lamiaceae, Magnoliaceae, Nelumbonaceae, Nymphaeaceae, Nyssaceae, Papaveraceae, Poaceae, Ranunculaceae, Solanaceae, and Typhaceae.

The serologic and disc electrophoretic characterization and comparison of the spore proteins extracted from *Osmunda cinnamomea*, *O. claytoniana*, and *O. regalis* illustrated that fern spores were suitable material for such analyses. *Osmunda cinnamomea* and *O. claytoniana* were shown to possess greater protein affinities for each other than either had for *O. regalis*. *Osmunda regalis*, in general, had greater protein affinities for *O. claytoniana* than it had for *O. cinnamomea* (Petersen & Fairbrothers, 1971). Stein & Thompson (1975) compared the same three *Osmunda* species using DNA hybridization techniques and independently indicated the same relationships reported by Petersen & Fairbrothers (1971). Miller (1967), based on anatomical characters of living and fossil specimens, indicated that *O. claytoniana* and *O. regalis* were more closely related, while Hewitson's (1963) anatomical and morphological research indicated that *O. cinnamomea* and *O. claytoniana* had a closer relationship with each other than either had with *O. regalis*.

The serological investigation of intra- and interfamily relationships of the Cornaceae and Nyssaceae has continued intermittently in our chemosystematics laboratory for 15 years, and various experiments have been conducted as appropriate and adequate plant materials became available. In our systematic serological research it has been expedient to conduct several projects simultaneously because no experiments can be conducted until adequate and appropriate materials are available for the extraction of proteins, and until antisera to perform the essential experiments have been raised.

Cornus canadensis and *C. suecica* have been found serologically very similar based on photronreflectometer tests, Ouchterlony plates, and absorped and non-absorbed antisera. These two taxa have also been shown to be the most dissimilar from other taxa placed in the genus *Cornus* (Fairbrothers, 1966a, 1966b, 1968). When data were evaluated from cytology, morphology, anatomy, geographical distribution, and the putative hybrid (*C. unalaschkensis*), the close similarity between the two was also detected. I believe all the data indicate that the two named taxa are subspecies of one circumboreal species which is very distinct from the other species of *Cornus*. If there is justification for dividing the genus *Cornus* into distinct genera, then this species (or two species) would

TABLE 1.[a] Data obtained from precipitin reactions with species of *Camptotheca* (Ca.), *Cornus* (C.), *Corokia* (Co.), *Davidia* (D.), and *Nyssa* (N.) using the photronreflectometer. The numbers represent percent area of the reference reaction, which is expressed as 100%.

	Antisera[b]										
Antigens	*C. amomum* R-110[5]	*C. amomum* R-118[4]	*C. canadensis* R-106[5]	*C. racemosa* R-124[4]	*C. racemosa* R-125[5]	*N. aquatica* R-98[4]	*N. aquatica* R-111[3]	*N. ogeche* R-112[4]	*N. sylvatica* R-114[4]	*N. sylvatica* R-116[5]	*D. involucrata* R-105[4]
C. amomum	100	100	50	90	84	56	32	32	27	47	—
C. stolonifera	81	—	—	—	—	—	—	—	—	—	—
C. racemosa	73	84	49	100	100	57	33	28	26	45	—
C. florida	54	—	—	—	65	—	—	—	—	—	—
C. kousa	48	—	—	—	60	—	—	—	—	—	—
C. nuttallii	50	—	—	—	62	—	—	—	—	—	—
C. canadensis	—	38	100	—	46	35	—	15	—	28	—
Ca. acuminata	30	33	25	34	38	69	50	47	44	65	39
N. aquatica	36	26	15	—	30	100	100	76	66	92	—
N. ogeche	—	26	12	—	20	89	—	100	—	86	—
N. sylvatica	33	31	15	34	20	94	83	83	100	100	52
D. involucrata	23	—	—	31	—	—	41	—	34	—	100
Co. cotoneaster	7	—	—	3	10	—	10	10	4	—	5

[a] It is essential that these new data be evaluated with previous published photronreflectometer data for some of the included taxa (Fairbrothers & Johnson, 1964; Fairbrothers, 1966a, 1966b, 1968).

[b] The superscripts on the antisera numbers indicate the number of injection series each experimental animal received.

TABLE 2.[a] Number of immunoprecipitating systems (bands) obtained from Ouchterlony Plates (double diffusion) for *Cornus* (C.), *Camptotheca* (Ca.), *Corokia* (Co.), *Davidia* (D.), and *Nyssa* (N.). Seven antisera are compared with nine antigens.

	Antisera[b]																				
	C. amomum R-110[5]			*C. canadensis* R-106[5]			*C. racemosa* R-124[4]			*N. aquatica* R-98[4]			*N. ogeche* R-112[4]			*N. sylvatica* R-116[5]			*D. involucrata* R-105[4]		
	Banding Patterns[c]																				
Antigens	I	N	T	I	N	T	I	N	T	I	N	T	I	N	T	I	N	T	I	N	T
C. amomum	5	0	5	1	1	2	2	1	3	1	1	2	0	2	2	1	1	2	1	1	2
C. racemosa	2	1	3	1	1	2	4	0	4	1	1	2	0	2	2	1	1	2	1	1	2
C. canadensis	0	2	2	4	0	4	1	1	2	0	2	2	0	1	1	0	2	2	0	2	2
Ca. acuminata	1	1	2	0	1	1	1	1	2	1	2	3	1	1	2	2	1	3	2	1	3
N. aquatica	1	1	2	0	1	1	0	2	2	4	0	4	3	0	3	3	0	3	2	1	3
N. ogeche	1	1	2	0	1	1	0	2	2	2	1	3	4	0	4	2	1	3	—	—	—
N. sylvatica	1	1	2	0	1	1	0	1	1	3	1	4	2	1	3	5	0	5	2	1	3
D. involucrata	—	—	—	—	—	—	1	1	2	—	—	—	—	—	—	1	2	3	4	0	4
Co. cotoneaster	0	1	1	0	1	1	0	1	1	0	2	2	0	1	1	0	1	1	0	2	2

[a] It is essential that these new data be evaluated with previous published serological data for some of the included taxa (Fairbrothers & Johnson, 1964; Fairbrothers, 1966a, 1966b, 1968).
[b] The superscripts on the antisera numbers indicate the number of injection series each experimental animal received.
[c] Banding patterns have been designated as follows: I = identity bands, N = nonidentity and/or partial identity bands, T = total number of bands.

best qualify for such a designation, and would have to be given the generic name of *Chamaepericlymenum.*

Serological data have also indicated that within the genus *Cornus* there are the following three distinct groupings: (1) *C. florida*, *C. kousa*, and *C. nuttallii*; (2) *C. amomum*, *C. stolonifera*, and *C. racemosa*; and (3) *C. canadensis* and *C. suecica* (Fairbrothers & Johnson, 1964; Fairbrothers, 1966a, 1966b, 1968). These serological groupings correspond to the *Cornus* subgenera designated by Ferguson (1966), except he placed *C. kousa* in a subgenus distinct from that containing *C. florida* and *C. nuttallii.* Newer serological data from our laboratory based upon additional antisera and many more experiments continue to support a tripartite taxonomic disposition of the taxa placed in the genus *Cornus* (Tables 1–2). The use of nonflavonoid glucosides as taxonomic markers in the genus *Cornus* was reported by Jensen et al. (1975). Their suggested arrangement of subgenera based on the presence or absence of iridoids, plus the type of iridoid constituents agree with the reported serological groupings and would correspond to their (A/B), (C), and (F/G/H) designations.

The families Cornaceae and Nyssaceae were recognized by Dumortier in 1829. However, this separation into two families was not followed by most taxonomists for over 100 years. Recently Melchior (1964), Cronquist (1968), Thorne (1968, 1976), Takhtajan (1969), and Dahlgren (1975) have recognized two families. In addition, in most recent classifications the genus *Davidia* has been removed from the Nyssaceae and placed in the Davidiaceae (Melchior, 1964; Cronquist, 1968; Takhtajan, 1969; Dahlgren, 1975). Thorne (1968, 1976) placed *Davidia* in the subfamily Davidioideae of Nyssaceae following Wagerin (1910). Harms (1898) was the first author to use the two subfamilies Davidioideae and Nyssoideae, and he placed them both in the family Cornaceae.

The serological data support the separation of the Cornaceae and Nyssaceae, and the grouping of *Camptotheca*, *Davidia*, and *Nyssa* within the Nyssaceae (Tables 1–2). At present I believe the serological data best support the placement of *Davidia* in the Davidioideae, and *Camptotheca* and *Nyssa* in the Nyssoideae both of the family Nyssaceae (Fairbrothers & Johnson, 1964; Fairbrothers, 1966a, 1966b, 1968; Tables 1–2).

Perdue et al. (1970) reported that tests with *Camptotheca acuminata* demonstrated that crude extracts exhibited significant activity against lymphoid leukemia. This comprehensive report discussed the relationships of *Camptotheca* within the Nyssaceae, indicating that *Camptotheca* was closely related to *Nyssa* and only remotely related to *Davidia.* Research done by Titman (1949) using wood anatomy, Eyde (1963) using fruit structure and the fossil record, and Sohma (1963) using pollen support the taxonomic conclusions of Perdue et al. (1970). Our recent serological data also indicate that *Camptotheca* is more similar to *Nyssa* than to *Davidia*, and that *Nyssa* is more similar to *Davidia* than is *Camptotheca* (Tables 1–2).

Our serological data are also supported in part by the findings of Hohn & Meinschein (1976) based on the fatty acid composition of seeds. They indicated that primitive *Davidia* and advanced *Camptotheca* were placed on each side of *Nyssa*, which is intermediate. Thus all the data presented lend credence

to the placement of *Camptotheca* and *Nyssa* in the subfamily Nyssoideae and *Davidia* in the Davidioideae of the Nyssaceae.

Serological experiments with four species of *Nyssa* have included comparisons of *Nyssa aquatica*, *N. biflora*, *N. ogeche*, and *N. sylvatica*. The various experiments indicated that *N. biflora* and *N. sylvatica* were serologically very similar. *Nyssa ogeche* and *N. aquatica* were serologically distinct from each other and from *N. biflora* and *N. sylvatica*. The data also showed that serologically *N. aquatica* was more similar to *N. biflora* and *N. sylvatica* than to *N. ogeche*. However, *N. ogeche* was more similar to *N. aquatica* than to *N. biflora* and *N. sylvatica*. *Nyssa ogeche* was the most distinct species of the four compared (Fairbrothers & Johnson, 1964; Fairbrothers, 1966a, 1966b, 1968; Tables 1–2). The serological data support the conclusions of both Eyde (1963) and Sohma (1963), who reported close similarity between *N. biflora* and *N. sylvatica* and treated them as two varieties of one species. The serological data does not support the findings of Hohn & Meinschein (1976) based on seed oil fatty acids. They indicated *N. biflora* and *N. sylvatica* to be chemically distinguishable species. The various researchers agree that *N. ogeche* is the most distinct from the other three species of *Nyssa*. The serological data has not clearly indicated whether *Camptotheca*, *Davidia*, or *Nyssa* has the greatest similarity with *Cornus*. The three genera are serologically relatively similar to *Cornus*; however, the data indicate that *Camptotheca* might have slightly more similarity with *Cornus* than do *Nyssa* or *Davidia* (Tables 1–2).

The genus *Corokia* (6 species) is restricted to the South Pacific region, ranging from northern New South Wales, Lord Howe Island, New Zealand, Chatham Islands, and Rapa Island, a distance of 4,000 miles.

The serological data reveal very little similarity between *Corokia cotoneaster* and any species of the Cornaceae and Nyssaceae tested (Fairbrothers et al., 1975; Tables 1–2).

Most researchers have indicated that this genus has little affinity with members of the Cornaceae in which it is often placed. Some botanists have suggested an affinity with the Saxifragaceae within the subfamily Escallonioideae (Escalloniaceae) (Philipson, 1967; Smith, 1958). Eyde (1966, 1967) concluded that it was unrelated to *Cornus* but was possibly linked with *Argophyllum*. Kubitski (1963) retained the genus in the Cornaceae. Hegnauer (1965) indicated that the Cornaceae may be related to either the Saxifragaceae or Loganiaceae. Takhtajan (1969) excluded the genus *Corokia* from the Cornales and placed it in the Escalloniaceae (Saxifragales). Cronquist (1968) considered *Corokia* as a possible nonmissing link between the Cornaceae and Escalloniaceae, Grossulariaceae, or Saxifragaceae sensu lato. Both Cronquist's and Takhtajan's classifications reflect the serological data which indicate the distinctiveness of *Corokia* from members of the *Cornales*.

However, Bate-Smith et al. (1975) investigated the distribution of several chemical compounds in the Cornales and concluded that *Corokia* possesses a chemical pattern consistent with that of the Cornaceae.

The experimental investigation of taxa within the Cornales has indicated that the use of diverse disciplines has provided valuable data for helping to

understand the evolutionary development and relationships of the families and genera in the order. This order has proven to have been an excellent one for diverse chemosystematic research. Serological comparisons have provided significant data for evaluation in the continuing investigation of diverse cornaceous, sensu lato, taxa.

Taxonomy eventually must strive to bring together, summarize, and utilize what is known about the organisms to be compared. Systematic serologists have essentially learned to use the properties of one of the classes of proteins, gamma globulins, in comparative studies. Systematic serology provides comparisons which are relatively objective measurements; however, like all detected relationships, they are relative and not absolute. Thus, as stated in the first paragraph of this paper, I hope that the included information has helped the reader to formulate perspectives concerning plant serotaxonomic research.

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